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(54) Title: ANTI-BACTERIAL COMPOSITIONS

(57) Abstract

The present invention provides a method for preventing a microorganism from growing on surfaces such as the skin, walls, floors, countertops, and food preparation surfaces as well as in absorbent materials such as diapers, clothing, bedding, bedpads, surgical apparel, surgical masks and the like. This method includes incorporating an effective amount of zeolite onto the surface or into the absorbent material to inhibit such a microorganism from growing.

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ANTI-BACTERIAL COMPOSITIONS

FIELD OF THE INVENTION:

5   The present invention relates to novel methods for preventing microbial growth and the breakdown of urea to ammonia in absorbent materials and on surfaces such as, for example, diapers, clothing, bedsheets, bedpads, blankets, filters, filtering aides, wall coverings, countertops,
10   cutting boards, and the like. These methods involve contacting the microorganism with an absorbent material or a surface which contains an effective amount of a zeolite.

BACKGROUND OF THE INVENTION:

15   Bacteria growth can lead to disease, particularly when the species of bacteria is pathogenic. Even when a bacterial species is not normally pathogenic, a person or animal which is immunologically compromised may become infected with that bacterial species. While antibiotics
20   are often used to combat such bacterial infections, bacteria can become resistant to those antibiotics and excessive use of antibiotics is frequently blamed for the occurrence of antibiotic-resistant bacteria. Moreover, many people are allergic to certain types of antibiotics
25   and antibiotic usage can cause gastrointestinal upset or other side effects. For example, more than 75% of infants prescribed Amoxycillin™ or Augmentin™ develop diarrhea, which causes a four-fold increase in the probability of developing a diaper-area dermatology disease. Similarly,
30   agricultural animals are healthier and more productive when bacterial exposure is reduced but antibiotic usage, with its attendant side effects and tendencies to give rise to antibiotic-resistant strains of bacteria, has been the only effective procedure for accomplishing these results.
35   Hence, new methods are needed for preventing exposure of mammals to bacteria and for inhibiting bacterial growth once such exposure has occurred.
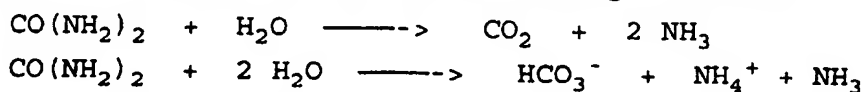
For example, methods for reducing bacterial growth in
and on materials which are used near or by hospital
patients, infants, the elderly and other persons whose
immune systems are weakened, would greatly reduce the need
5   for antibiotic usage. Similarly, methods for inhibiting
bacterial growth in materials which contact the skin of
such persons can prevent dermatological problems. Methods
for inhibiting bacterial growth on surfaces used for food
preparation would reduce the incidence of food poisoning.
10   Methods of reducing bacterial growth in the litter, bedding
and even the soil where domesticated animals are maintained
would reduce animal mortality and increase yields of eggs,
meat and milk.

Some materials are known to support bacterial growth,
15   for example, the urine and feces captured by a diaper, but
no method currently exists for inhibiting such growth.
Even though it is initially sterile, bacteria and other
microbes frequently begin to grow in urine and, when the
skin is exposed to urine for any length of time,
20   dermatological problems can result. Similarly, fecal
material is rife with microorganisms that can cause
dermatological problems, e.g. by producing proteolytic
enzymes and lipases. These dermatological problems can
also be created or exacerbated by ammonia formed by the
25   breakdown of urea. The skin and tissues are irritated by
ammonia at concentrations of 10,000 ppm; higher exposures
produce burns and blistering. Adult tissues and moist
membranes are irritated by ammonia gas at concentrations as
low as 35 ppm for 15 minutes. See Proctor et al., Chemical
30   Hazards of the Workplace (2d ed. 1988); 2B Patty's
Industrial Hygiene and Toxicology 3045 (1981); Clayton and
Clayton, Safety Standards for Industrial Chemicals (1981);
NIOSH, Criteria for Recommended Standard Exposure to
Ammonia (1981); American Conference of Governmental
35   Industrial Hygienists (1987). Moreover, ammonia gas can be
life-threatening to healthy adults at levels of about 1500
parts per million (ppm) over about two hours. The safe

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industrial limit of exposure to ammonia by adults is 35 to
50 ppm for 15 minutes. Breathing 5000 ppm can be
immediately fatal. Bacterial growth exponentially
increases the rate of ammonia formation.

5   Infants and the elderly can be even more sensitive to
dermatological problems and to the effects of ammonia. For
example, low birth weight infants often have underdeveloped
respiratory systems which may be especially vulnerable to
the toxic effects of ammonia. Infants and the elderly with

10 any form of pulmonary disfunction such as bronchopulmonary
dysplasia, chronic respiratory failure and pulmonary
hypertension may succumb to ammonia poisoning more easily
than healthy individuals.

For example, a baby urinates about 0.75 ml to 1.0 ml of

15 urine per hour per kilogram of bodyweight. Urine is about
96% water, 2% urea and 2% other materials. The excreted
urine passes through a membrane-sleeve of conventional
disposable diapers, which are highly effective at absorbing
water but not very effective at absorbing ammonia or

20 ammonium ions. Moreover, while water is held tightly, a
conventional diaper does not prevent microbial growth. In
the presence of such microbes, urea present from excreted
urine and feces begins to break down in minutes into
ammonia and ammonium, by the following formulae:

25      $CO(NH_2)_2 \ + \ H_2O \ \longrightarrow \ CO_2 \ + \ 2 \ NH_3$
        $CO(NH_2)_2 \ + \ 2 \ H_2O \ \longrightarrow \ HCO_3^- \ + \ NH_4^+ \ + \ NH_3$
The rate of _in vitro_ ammoniation increases hourly by 5.8%,
6.3%, 8.0% and 9.7% through hours 2 to 5 after voiding.
This steeply increasing rate of ammoniation is caused by

30 growing colonies of bacteria and the consequent
accumulation of urease which deaminates urea.

The pH of urine at the time of voiding is about 6.0,
but, as NH_4OH and NH_3 continually form over time the pH
rises. At about three hours the pH is about 9.0 and NH_3

35 gas flows continuously. While some microbial growth is
retarded by pH 6.0 urine, an increased pH stimulates
bacterial growth. Each urination provides fresh urea to

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start a new cycle of deamination. Ammonia is also formed by breakdown of microbial amino acids. This rising pH provides an increasingly hospitable environment for bacterial and other microbial colonies.

Moreover, even though the growth of microbes is momentarily slowed by a fresh voiding of pH 6.0 urine, such microbes quickly recover, continue growing and decompose more urea and amino acids.

Once ammonia gas is formed, it begins escaping back across a disposable diaper's membrane-sleeve where it contacts the skin and feces, if present. Ammonia elevates the pH of whatever medium it contacts. For example, the alkaline ammonia gas elevates the fecal pH which activates proteolytic enzymes that vigorously metabolize skin, causing irritations, rashes or lesions. Such damage makes the skin more vulnerable, not only to ammonia, but to opportunistic microbes, including fungi such as _Candida_, and bacteria that normally do not inhabit the skin.

When disposable diapers become saturated with urine, pressure of 0.5 psi will force urine back through the membrane-sleeve where it contacts skin and feces, a phenomenon known as "re-wetting." Frequently, disposable diapers become saturated and the membrane-sleeve bursts open, permitting urine to contact skin and feces. In either case, this urine is aged or ripe and contains deaminating urea, ammonium, ammonium hydroxide, dissolved ammonia, ammonia gas and carbon dioxide, as well as a potentially enormous inoculum of urea-cleaving bacteria. Large numbers of bacteria can be toxic, especially if they enter body cavities or if subdermal areas of the epidermis have been breached. The carbon dioxide is known to be toxic when breathed, and is believed to cause fatal hypercarbia to infants in certain conditions. _See_ Thach, J. PEDIATRICS (June, 1993); Kemp & Thach, PEDIATRIC RES. (July 1994); Kinney et al. SCIENCE (Sept. 1995). A combination of ammonia gas and carbon dioxide is believed to be more toxic than either gas alone.

According to the present invention, zeolites prevent microbial growth thereby inhibiting urea from converting to ammonia and carbon dioxide. Therefore, zeolites have two beneficial effects: inhibition of bacterial growth and inhibition of ammonia and carbon dioxide production.

Zeolites can be chemically synthesized and also occur naturally in volcanic rocks, altered basalts, ores and clay deposits. Zeolites include crystalline, hydrated alkali-aluminum silicates of the general formula:

$$M_{2/n}O \cdot (Al_2O_3) \cdot [y(SiO_2)] \cdot wH_2O$$
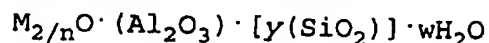
wherein M is a cation of valence n, w is the number of water molecules, and y is 2 or more. The cation is mobile and can undergo ion exchange. See U.S. Patent 2,882,243 to Milton.

Zeolites have been used as catalysts, adsorbents and ion exchange media in chemical and hydrocarbon processing procedures. Some forms of crystalline aluminosilicate zeolites are regenerated after use in such procedures, often by acid treatment or thermal treatment at very high temperatures. Resistance to such treatment is related to the presence of a higher proportion of SiO_2 relative to Al_2O_3 in the aluminosilicate. See U.S. Patent 3,691,099 to Young.

Crystalline, hydrated aluminous tectosilicates of Group I and II elements such as potassium, magnesium and calcium are also formed in nature or may be synthesized in the laboratory. Higher polyvalent ions, such as the rare earths, are readily introduced by cation exchange. Structurally, these tectosilicates form an aluminous silicate "framework" extending as an infinite three-dimensional network of AlO_4 and SiO_4 tetrahedra linked together by shared oxygen atoms. These aluminous tectosilicates are represented by the empirical unit cell formula:

$$M_{x/n}[(AlO_2)_x(SiO_2)_y]wH_2O$$
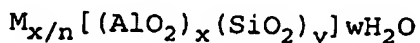
wherein M is a cation of valence n, w is the number of water molecules, x is the number of AlO_2 units and y is the

number of SiO_2 units. The ratio of y/x is usually about 1
to about 10. The sum of x and y is the total number of
tetrahedra in the unit cell.

Channels and pores uniformly penetrate the entire
volume of the solid zeolite. When water is removed from
these zeolites, large internal surface areas become
available to absorb liquids or gases. Thus, the external
surface area of a zeolite represents only a small portion
of its total available surface area. Moreover, the
dehydrated zeolite selectively absorb or reject different
molecules on the basis of their effective molecular sizes
and shapes.

Point electric charges on the surfaces of aluminous
zeolite pores absorb highly polar molecules such as water,
alcohols and the like. Such hydrophilicity has been
exploited to remove water from polar substances which are
less readily absorbed by the aluminous zeolite, for
example, hydrocarbons processed by the petroleum industry.
Gas streams may also be dried with a dehydrated zeolite due
to its extremely strong attraction for water. Both
naturally-occurring and synthetically-prepared zeolites
have been used to remove nitrogenous components from liquid
human and animal wastes by ion exchange. See U.S. Patent
No. 3,935,363 to Burholder. Metal catalysts have been
introduced into zeolites for converting carbon monoxide to
carbon dioxide or for catalyzing the hydrogenation and
cracking of petroleum feedstocks. See British Patent No.
2,013,476A. Hydrophobic tectosilicates, developed to
resist water absorption, will absorb less polar substances
from mixtures containing water. See U.S. Patent Nos.
4,744,374 and 4,683,318. For example, U.S. Patent No.
3,682,996 to Kerr disclosed silylation of free hydroxy
sites in zeolites by trimethylsilane ($H-Si(CH_3)_3$) and that
such silylated zeolites absorbed about 40% less
cyclohexane, n-hexane and water than the parent "hydrogen"
zeolites of type II. However, Kerr did not report any
change in selectivity preference. Similarly, R. M. Barrer

and J.-C. Trombe, J.C.S. Faraday I, 74, 1871 (1978), reported some nest silylation to form a tectosilicate of structure V (R=SiH$_3$, x<4) but were largely unsuccessful in replacing lattice aluminum with silicon, reporting that nest hydroxyl groups appeared to be less reactive to silylation than are the hydroxyl groups of the present structure II.

However, there has been no recognition of the present beneficial properties of either hydrophilic or hydrophobic zeolites for preventing microbial growth and for preventing the conversion of urea and amino acids to ammonia. Instead, skin rashes and irritations are frequently treated with salves and ointments. For example, U.S. Patent 4,556,560 to Buckingham provides a lipase-inhibiting agent which is preferably applied with a barrier-like vehicle for treatment of diaper rash and diaper dermatitis. Medicated greases such as Balmex® and A&D Ointment® are also widely available. However, while these products may provide some protection from ammonia burns, they also block oxidation of the injured skin and create an ideal environment for growth of anaerobic bacteria. Therefore, a long-standing need exists for a new solution to dermatological problems such as diaper rash and dermatitis.

SUMMARY OF THE INVENTION:

The present invention provides a method for preventing a microorganism from growing in a diaper, clothing, bedding or a bedpad by incorporating an effective amount of zeolite into the diaper, clothing, bedding or bedpad to inhibit the microorganism from growing.

This and other objectives of the present invention are more specifically described in the detailed description of the invention, provided hereinbelow.

BRIEF DESCRIPTIONS OF THE DRAWINGS:

Fig. 1 depicts procedures for derivatization of aluminous tectosilicates. Starting materials are depicted by formula I:

$$(\equiv Si-O)_4\ Al\_M^+ \qquad\qquad I$$

wherein M^+ is a metal cation. These starting materials are
treated with acid to provide aluminum-associated hydroxyl
sites on the tectosilicate of formula II:

$$(\equiv Si-O)_3\ Al\ +\quad HO-Si\equiv \qquad II$$
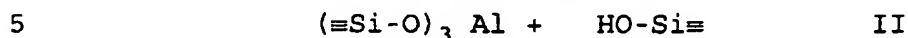
5 Exposure to a silylating agent, for example
trimethylsilane, provides a silylated, aluminum-containing
material of formula III:

$$(\equiv Si-O)_3\ Al^+(CH_3)_3\ Si-O-Si\equiv \qquad III$$
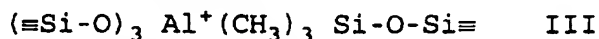
10 Alternatively, extended treatment of aluminous
tectosilicates with aqueous acid provides tectosilicates
with a high proportion of silicon to aluminum. These
dealuminated tectosilicates contain tetracoordinated
hydroxylated nests of about four $\equiv Si-OH$ moieties, as

15 depicted by formula IV. Heating at low temperatures of
about 100°C to 200°C clears pores and channels in the
tectosilicates by removing water of hydration. Treatment
with a derivatizing reagent, RX, yields derivatized,
dealuminated tectosilicates of formula V:

20 $$(\equiv Si-OR)_x \qquad\qquad IV$$
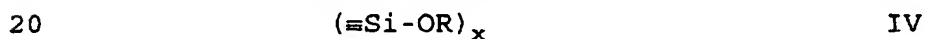
wherein R is alkyl, acyl, or silyl substituted with 1-3
halogen, alkoxy alkyl, aryl, aralkyl or cycloalkyl
substituents.

Fig. 2 depicts the log reduction of Staphlococcus
25 aureus cell titer caused by several dilutions of a 45 mg/ml
clinoptilolite derivatized with dichlorodimethylsilane
(DCDMS Zeolog). The log reduction is log (initial cell
titer/final cell titer), where the initial titer was 9.2 X
10^9 colony forming units per ml of culture (cfu/ml). As

30 illustrated, all zeolog dilutions between 10^{-1} and 10^{-5}
reduced the Staphlococcus aureus cell titer, but a dilution
of 10^{-1} almost completely inhibited cell growth (by causing
a 3 X 10^9 cfu/ml reduction in cell titer).

Fig. 3 depicts the log reduction of Staphlococcus
35 aureus cell titer caused by several dilutions of a 45 mg/ml
clinoptilolite derivatized with methyltriethoxy groups
(PQ99 Zeolog, also known as ZeoLog-MeTE). The log

reduction is log (initial cell titer/final cell titer),
where the initial titer was 9.2×10^8 cfu/ml. As
illustrated, zeolog dilutions between 10^{-1} and 10^{-2} reduced
the <u>Staphlococcus</u> <u>aureus</u> cell titer, but a dilution of 10^{-1}
5   caused more cell growth inhibition (about a 2×10^8 cfu/ml
reduction in cell titer).

Fig. 4 depicts the log reduction of <u>Staphlococcus</u>
<u>aureus</u> cell titer caused by several dilutions of a 45 mg/ml
clinoptilolite derivatized with methanol (MeOH Zeolog, also
10   known as ZeoLog Methanol). The log reduction is log
(initial cell titer/final cell titer), where the initial
titer was 9.6×10^8 cfu/ml. As illustrated, zeolog
dilutions between 10^{-1} and 10^{-5} completely inhibited
<u>Staphlococcus</u> <u>aureus</u> cell growth, causing a 6×10^8 cfu/ml
15   reduction in cell titer.

Fig. 5 depicts the log reduction of <u>Escherichia</u> <u>coli</u>
cell titer caused by several dilutions of a 45 mg/ml
clinoptilolite derivatized with dichlorodimethylsilane
(DCDMS Zeolog). The log reduction is log (initial cell
20   titer/final cell titer), where the initial titer was $7.9 \times$
10^8 cfu/ml. As illustrated, zeolog dilutions between 10^{-1}
and 10^{-2} completely inhibited <u>Escherichia</u> <u>coli</u> cell growth,
the 10^{-3} dilution caused a 1×10^7 cfu/ml reduction in cell
titer, and the 10^{-4} dilution caused a 1×10^2 cfu/ml
25   reduction in cell titer.

Fig. 6 depicts the log reduction of <u>Escherichia</u> <u>coli</u>
cell titer caused by several dilutions of a 45 mg/ml
clinoptilolite derivatized with methyltriethoxy groups
(MTTXY-Zeolog, also known as ZeoLog-MeTE). The log
30   reduction is log (initial cell titer/final cell titer),
where the initial titer was 7.9×10^8 cfu/ml. As
illustrated, zeolog dilutions between 10^{-1} and 10^{-2}
inhibited <u>Escherichia</u> <u>coli</u> cell growth, by 9×10^7 and $9 \times$
10^2 cfu/ml, respectively.

35   Fig. 7 depicts the log reduction of <u>Salmonella</u>
<u>typhimurium</u> cell titer caused by several dilutions of a 45
mg/ml clinoptilolite derivatized with dichlorodimethyl-

silane (DCDMS Zeolog). The log reduction is log (initial cell titer/final cell titer), where the initial titer was 3.6×10^9 cfu/ml. This type of derivatized zeolite provided little reduction in Salmonella typhimurium cell

5   titer.

Fig. 8 depicts the log reduction of Salmonella typhimurium cell titer caused by several dilutions of a 45 mg/ml clinoptilolite derivatized with methyltriethoxy groups (MTTXY Zeolog, also known as ZeoLog-MeTE). The log

10   reduction is log (initial cell titer/final cell titer), where the initial titer was 7×10^9 cfu/ml. As illustrated, zeolog dilutions of 10^{-3} and 10^{-4} reduced the Salmonella typhimurium cell titer by 9×10^{-2} and 4×10^5 cfu/ml, respectively.

15   Fig. 9 depicts the log reduction of Salmonella typhimurium cell titer caused by several dilutions of a 45 mg/ml clinoptilolite derivatized with methanol (MeOH Zeolog, also known as ZeoLog Methanol). The log reduction is log (initial cell titer/final cell titer), where the

20   initial titer was 2.1×10^9 cfu/ml. As illustrated, 10^{-4} and 10^{-5} dilutions of a 45 mg/ml suspension of methanol-derivatized zeolite effectively reduced the Salmonella typhimurium cell titer by about 2×10^4 and 4×10^5 cfu/ml, respectively.

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DETAILED DESCRIPTION OF THE INVENTION:

According to the present invention, microbial growth is inhibited by zeolites. Use of zeolites therefore prevents bacterial infections and rashes in mammals, for example,

30   when used in materials that may contact hospital patients, in surfaces used for food preparation, in diapers, in bedding, in bedpads and the like. Prevention of bacterial growth also prevents ammonia formation which can cause or exacerbate skin rashes.

35   The methods of the present invention prevent microorganisms from growing, for example, on the skin, in diapers, in clothing, in bedding, in bedpads, in

wallcoverings, in paint, in surgical apparel, in surgical
masks, on countertops, in cutting boards and the like.
Such microorganisms grow on these materials particularly
when food, urine, feces, perspiration and the like are
present.

The present invention contemplates methods of
inhibiting growth and urea breakdown by microorganisms
commonly found on the skin and in decaying food, urine,
perspiration and feces. Growth of bacteria commonly found
in hospitals can also be inhibited. Such microorganisms
include members of the family Enterobacteriaceae as well as
a large number of gram positive microbes. Gram negative
Enterobacteriaceae which are included in the present
invention are members of the genus Proteus, including P.
mirabilis, P. vulgaris, P. penneri and P. myxofaciens.
Other genera contemplated by the present invention include
Klebsiella, Providencia, Yersinia, Erwinia, Enterobacter,
Salmonella, Serratia, Aerobacter and Escherichia. In
addition, related gram negative genera fall within the
scope of the invention, including Pseudomonas, Shegella,
Vibrio, Aeromonas, and Campylobacter.

The gram positive microorganisms falling within the
scope of the invention include the following genera,
Streptococcus, Staphylococcus, Lactobacillus, Micrococcus
and the Moraxella-Neisseria group.

The present methods are especially effective for
preventing growth of Escherichia coli and Staphylococcus
aureous.

Moreover, the present methods are effective for
preventing growth of ammonia-producing microorganisms, e.g.
those disclosed in J.J. Leyden, Urinary Ammonia and
Ammonia-Producing Microorganisms in Infants With and
Without Diaper Dermatitis, 113 ARCHIVES OF DERMATOLOGY (Dec.
1977). Such ammonia-producing microorganisms include the
aerobic diptheroids found on human skin, Corynebacterium
and Brevibacterium ammoniagenes.

The present methods are practiced on both humans and animals. In a preferred embodiment, the present methods are used for hospital patients, for infants, for the elderly, for farm animals and for food preparation. In an especially preferred embodiment, the present methods are used for treating infants and the elderly who have skin irritations, rashes or lesions.

While not wishing to be limited thereby, the methods of the present invention may compromise cell wall processes including basic transport processes. For example, zeolites may capture or neutralize electrons and inhibit electron transport through key enzymes of the electron transport chain such as cytochrome oxidase.

Both naturally-occurring and synthetic zeolites are used in the methods of the present invention. Both aluminated and de-aluminated zeolites are contemplated by the present invention, but, in one embodiment, dealuminated zeolites are preferred. Dealuminated zeolites have interior acid sites provided by protons which are left when the aluminum is removed. These interior acid sites react with, and hold cations such as ammonia. Because the interior acid sites are inside the zeolite materials, these acid sites do not touch the skin. In another embodiment, preferred zeolites are derivatized with substituents that are weaker point electric sources than hydroxy. Such dealuminated and derivatized zeolites not only bind ammonia in the presence of water but also inhibit microbial growth.

Synthetic zeolites for use in the present methods include zeolites derivatized with dichlorodimethyl silane, ZeoLog-MeTE, ZeoPhob™, ZeoLog™, ZeoLogCN-METHANOL™, Zeolite A (see U.S. Patent 2,882,243); Zeolite B (see U.S. Patent 3,008,803); Zeolite D (see Canada Patent No. 611,981); Zeolite E (see Canada Patent No. 636,931; Zeolite F (see U.S. Patent No. 2,995,358); Zeolite H (see U.S. Patent No. 3,010,789); Zeolite J (see U.S. Patent 3,011,869); Zeolite KG (see U.S. Patent 3,056,654); Zeolite L (see Belgium Patent No. 575,117); Zeolite M (see U.S.

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Patent 2,995,423); Zeolite O (see U.S. Patent 3,140,252);
Zeolite Q (see U.S. Patent No. 2,991,151); Zeolite R (see
U.S. Patent 3,030,181); Zeolite S (see U.S. Patent
3,054,657); Zeolite T (see U.S. Patent 2,950,952); Zeolite
W (see U.S. Patent 3,012,853); Zeolite X (see U.S. Patent
No. 2,882,244); Zeolite Y (see U.S. Patent 3,130,007); and
Zeolite Z (see Canada Patent No. 614,995).

Naturally-occurring aluminosilicate zeolites which are
used in the present methods include analcite, brewsterite,
chabazite, clinoptilolite, dachiardite, datolite, erionite,
faujasite, ferrierite, flakite, gmelinite, harmotone,
heulandite, leucite, levynite, mesolite, mordenite,
natrolite, nepheline, noselite, paulingite, phillipsite,
scolecite, stilbite, and yugawaralite. Naturally-occurring
zeolites are preferred. A preferred naturally-occurring
zeolite is clinoptilolite.

The hydrophobic zeolites of the present invention are
prepared by removing a substantial proportion of the
aluminum from the lattice sites of a tectosilicate,
dehydrating the resulting aluminum-deficient tectosilicate
and substituting lattice silyl hydroxyl groups with organic
moieties which are weaker point electric sources than
aluminum. As used herein the term "weaker point electric
source" is a moiety which has a lower overall charge, or a
charge which is distributed over a larger molecular volume,
than the charge, for example, of an aluminum site in an
aluminous tectosilicate, or a hydroxyl site formed as a
result of aluminum removal. Preferred embodiments have
organic moieties which are weaker point electric sources
than hydroxy, such as acyl, alkyl, silyl and the like.
Preferably, the lattice silyl hydroxyl groups have a
tetracoordinate "nest" configuration. Preferred silyl
groups are those of the general formula $Si(R')_nX_p$ wherein n
is 0 to 3, p is 3-n, R' is selected from the group
consisting of aryl, alkyl, acyl, aralkyl, cycloalkyl and
mixtures thereof and X is halogen or an alkoxy group. The
preferred acyl, alkoxy and alkyl groups are lower acyl,

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alkoxy and alkyl radicals such as C_1-C_4 alkyl, alkoxy or
acyl groups, both branched and straight chain. Preferably,
n is 1-2 and X is chloro.

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The tectosilicates derivatized in this manner are
first dealuminated so as to produce the requisite reactive
sites in the silicaceous lattice. Preferably,
dealumination is accomplished by treatment with aqueous
mineral acid. The dealuminated tectosilicate is then
dehydrated to expose reactive lattice silyl hydroxyl
groups. Tectosilicates generally possess some hydroxyl
(OH) groups, but the number of hydroxyls is not sufficient
to allow derivatization to the extent necessary to impart a
useful degree of hydrophobicity. Accordingly, the
substrate tectosilicates is first treated to increase the
number of lattice hydroxyl groups, and then is dehydrated
to make the hydroxyl groups available for subsequent
chemical derivatization. Derivatization is by known
procedures, e.g. those provided in U.S. Patent Nos.
4,683,318 and 4,744,374 to Deffeyes, the disclosures of
which are incorporated herein by reference.

A general reaction scheme for derivatizing
tectosilicate zeolites is depicted in FIG. 1. Exposure of
tectosilicates to aqueous acids replaces the metal cation
(M+) of tectosilicates with a hydronium ion. As depicted
in FIG. 1, a Si-O-Al bond of starting material I readily
protonates and dissociates to provide aluminum-associated
hydroxyl sites within the lattice as shown by structure II.
These type II sites are silylated by known procedures, for
example as disclosed in U.S. Pat. No. 3,682,996 to Kerr or
U.S. Patent Nos. 4,683,318 to Deffeyes. In one embodiment,
such silylation is by exposure to trimethylsilane (H-
Si(CH$_3$)$_3$) to form silylated, aluminum-containing material
of structure III.

Aluminous tectosilicates having a silicon to
aluminum ratio (Si:Al) of greater than about 5 are almost
totally dealuminated without loss of lattice integrity.
See R. M. Barrer and M. B. Makki, 42 Can. J. Chem. 1481

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(1964). This is accomplished by extended treatment of aluminous tectosilicates with aqueous acid. Dealumination yields tectosilicates having tetracoordinated hydroxylated nests containing about 4 associated ≡Si-OH moieties, as depicted in structure IV. These aluminum-free sites are termed "exoaluminum sites".

Activated tectosilicate materials both of structure II and IV exhibit reduced hydrophilicity because of the absolute reduction of lattice charge from aluminum removal, but still absorb water via hydrogen bonding to hydrogen atoms associated with the remaining aluminum atoms and/or to the free silyl hydroxyl (SiOH) groups. Heating aluminum-containing or dealuminated tectosilicates to relatively low temperatures, i.e., to about 100°-200°C., preferably in the presence of a vacuum, clears pores and channels for absorption by removing water of hydration from the pores. However, exposure of dealuminated tectosilicates to higher temperatures, i.e., to about 400°-500°C., causes either partial or total destruction of the hydroxyl nests, via dehydroxylation and formation of new Si-O-Si bonds. Dealuminized mordenites having Si:Al ratios of greater than 80 will not absorb water vapor at a pressure of one or 12 mm of mercury. See N.Y. Chen in J. Phys. Chem. 80, 60 (1976).

The aluminous tectosilicates utilized as starting materials for preparing derivatized tectosilicates can include crystalline, amorphous and mixed crystalline amorphous tectosilicates of natural or synthetic origin or mixtures thereof. The water insoluble crystalline tectosilicates useful in the present invention are those that possess interstitial channels of a narrowest diameter of about 3-13Å. Hereinafter this diameter will be referred to as pore size.

A preferred pore size for the unmodified substrate materials which is useful in this invention is about 3-10Å, most preferably 3-8Å. The pore size of any given tectosilicate must be large enough to admit derivatization

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materials such as silanes, alcohols and the like, yet small
enough to prohibit entry of unwanted liquid or gas stream
components, i.e., aromatics, ketones, heterocyclic
compounds and the like. Tectosilicates possessing pore
sizes with the range of about 4-13Å readily admit small
gaseous elements and compounds such as water (kinetic
diameter $[\sigma]$ 2.65Å), carbon monoxide (σ=3.76Å), carbon
dioxide (σ-3.30Å) and ammonia (σ=2.60Å).

The most useful aluminous tectosilicate starting
materials preferably possess a lattice silicon to aluminum
ratio of greater than about 5:1. Tectosilicates having a
silicon to aluminum ratio of less than about five tend to
lose their structural integrity upon dealumination.

An especially preferred class of aluminous
tectosilicate starting materials is the naturally-occurring
clinoptilolites. These minerals typically have the unit
cell structure of the formula:

$$Na_6 [(AlO_2)_6 (SiO_2)_{30}] .24H_2O$$
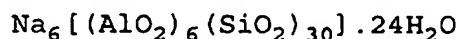
wherein the sodium ion content (Na+) is partially replaced
by calcium, potassium and/or magnesium, etc. The
silicon:aluminum ratio in preferred varieties is greater
than 5 and most preferably greater than about 8. The pore
size is in the range of about 3.0-6.0Å. Clinoptilolite is
stable in air to about 700°C and maintains its structural
integrity upon dealumination.

Other naturally-occurring aluminous tectosilicates
that are useful as starting materials are the mordenites,
which typically exhibit the unit cell composition:

$$Na_8 [(AlO_2)_8 (SiO_2)_{40}] .24H_2O$$
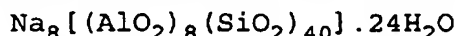
wherein calcium and potassium cations can replace a part of
the sodium cations. The pore size is in the range of about
3.5-4.5Å. The silicon to aluminum ratio is generally
greater than 5 but can be greater than 10. Other aluminous
tectosilicates such as ferrierite or erionite also provide
useful starting materials.

Although naturally-occurring aluminous
tectosilicates are acceptable starting materials, the

synthetic analogs of the natural tectosilicates and their derivatives are of equivalent utility in the present method. For example, synthetic mordenite (Zeolon®), available from the Norton Company, is an acceptable starting material for providing derivatized zeolites. Also, other synthetic, porous tectosilicates which have no equivalent in nature could serve as acceptable starting materials.

The formation of the hydrophobic materials of the present invention normally proceeds in three steps: (1) dealumination, (2) dehydration and (3) derivatization with an appropriate alkylating, acylating or silylating agent.

The dealumination of aluminous tectosilicates with acid is well known in the art. For example, R. M. Barrer & M. B. Makki (42 *Canadian J. Chem*. 1481 (1964)) reported the complete dealumination of clinoptilolite by refluxing samples in aqueous hydrochloric acid of varying concentration. In the present method, a strong acid treatment is preferred, involving exposing pulverized, sieved aluminous tectosilicate to refluxing, i.e. boiling 2-10N aqueous mineral acid for about 1-3 hours. Strong acids such as hydrochloric acid, sulfuric acid, nitric acid or phosphoric acid are preferably used. The more preferred acid is hydrochloric acid, at concentrations of about 3N to about 7N.

In some cases, dealumination is accomplished by a mild acid treatment involving the percolation of aqueous acid through a column of crushed aluminous tectosilicate under ambient conditions. Preferably, the tectosilicate starting material is dealuminated to achieve a Si:Al ratio of greater than about 25, but preferably the ratio exceeds 100, e.g. about 150-300. Under the most preferred conditions essentially no lattice aluminum is retained, as measured by X-ray fluorescence.

The dealuminated, air-dried tectosilicate materials are then heated in order to remove most of the pore water of hydration and to expose the remaining lattice

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hydroxyl groups to derivatization. The heating is carried
out at any temperature sufficient to effect substantial
dehydration without causing significant lattice
rearrangement and subsequent loss of reactive sites.

5   Typically, the dealuminated materials are heated to about
100°-200°C for about 10-40 hours, preferably under reduced
pressure. Derivatization after high temperature treatment
at about 500°-600°C, does not cause a further increase in
hydrophobicity for tectosilicates that had been subjected

10   to the acid treatment at ambient temperatures, although
substantial hydrophobicity is exhibited by the samples
relative to the starting materials.

            Following thermal dehydration at lower
temperatures, e.g., about 100°-200°C., the majority of

15   lattice silyl-hydroxyl groups which are present in
tetracoordinated nests of the unit structure IV, as
depicted in FIG. 1. The dealuminated, dehydrated materials
are cooled and then exposed to derivatizing reagents that
functionalize these internal lattice silyl-hydroxyl groups.

20   After exposure of the aluminum-free sites ("exoaluminum
sites") to the derivatizing reagent, the nest sites contain
from about 1-4 [Si-OR] units wherein R is alkyl, acyl, or
silyl, substituted with 1-3 halogen, alkoxy, alkoxy alkyl,
aryl, aralkyl or cycloalkyl substituents wherein the alkyl,

25   alkoxy or acyl groups, either directly attached to the
lattice silyloxy or bound to the silicon atom of the R
group, preferably are C_1-C_4 alkyl, alkoxy or acyl groups.

            Reagents (RX) useful to replace the hydrogen atom
of the nest silyl-hydroxyl groups with the substituent R

30   include a wide range of the reagents known in organic
chemistry to be useful to alkylate, acylate or silylate
hydroxyl groups. Such agents are generally disclosed by I.
T. Harrison et al. in *Compendium of Organic Synthetic
Methods*, Wiley-Interscience, N.Y. (1971) at pages 124-131,

35   the disclosure of which is incorporated by reference
herein.

Preferred reagents include the lower-C_1-C_4-alkanols, C_1-C_4 alkyl or C_1-C_4 alkyl halides such as methanol, ethanol and the like or methyl chloride, methyliodide, ethyl chloride, butyl bromide and the like. Lower-C_1-C_4-alkanols have been found to be especially effective as derivatizing agents when thermally reacted with the tectosilicates under pressure either neat or in the presence of catalysts. Other useful alkylating reagents include the C_1-C_4-diazo-alkanes.

In one embodiment, nest hydroxyl groups are acylated by exposure to ketenes such as ketene itself or to alkyl or dialkyl ketenes such as dimethylketene. Reaction of a silyl-hydroxyl group with ketene yields an SiOR moiety wherein R is acetyl, aralkyl and mixtures thereof; and X is a halogen atom, i.e., Cl, F, I, Br or mixtures thereof, or a (lower) alkoxy group.

Di-, tri- or tetra-functional silylation reagents also react with two to four $\equiv$Si-OH groups in a single nest to functionally replace the missing aluminum atom with the bridging unit SiR'_q, wherein q is 0-2, thus bridging the aluminum-deficient site with 1-2 silicon atoms. This reaction occurs via the elimination of 2-4 HX groups and the formation of O--Si--O bridges. For example, when dimethyldichlorosilane is reacted with lattice silyl hydroxyl groups ($\equiv$Si-OH), structural units such as $\equiv$SiOSi$(CH_3)_2$Cl or $\equiv$SiOSi$(CH_3)_2$OSi are introduced into the nests. The methyl groups are replaced with any of the groups represented by R' and Cl is replaced by another halogen atom or by an alkoxy group.

Typical monofunctional silylating reagents which introduce Si$(R')_3$ units include trimethylchlorosilane, trimethylflurosilane, dimethyliso-propyl-chloro-silane and the like. Preferred dysfunctional silylating agents include the dihalodialkylsilanes, e.g., dichlorodimethylsilane and the dialkoxy(dialkyl) silanes, e.g., diethoxy-dimethylsilane. Tri- and tetra-functional silanes are also employed to derivatize the tectosilicates

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of the present invention, such as silicon tetrafluoride, tetrachlorosilane, and trifluoromethylsilane.

Reaction of the dealuminated, dehydrated tectosilicates with the silylation reagent is carried out by contacting the materials with the reagent in the liquid or gas phase. Preferably, an excess of silylation reagent in a suitable solvent is slurried with the tectosilicate. Heating and/or added catalysts are employed if necessary, depending on the reactivity of the tectosilicate and the silane.

Gaseous hexamethyldisilazane is reacted with lattice hydroxyl groups to introduce trimethylsilyl groups into the nests, following the procedure of Fenimore et al., *Anal. Chem.*, 48,2289 (1976), the disclosure of which is incorporated herein by reference.

These procedures readily afford hydrophobic microporous, crystalline silaceous materials which exhibit a greatly reduced affinity for water while maintaining high affinities for less polar molecules such as ammonia. The hydrophobicity or reduction in hydrophilicity of a tectosilicate is quantitated in terms of its absorption of water per unit of tectosilicate under a given set of exposure conditions (retention volume). That any observed reduction in water retention is due to hydrophobicity as opposed to a general reduction in retention is established by measuring the retention of a similarly-sized molecule of comparable or lesser polarity, such as ammonia, nitrogen, methane or carbon dioxide.

Microporous crystalline silaceous materials with reduced affinity for water vapor are useful in the methods of the present invention. The tectosilicates are acid treated and activated for derivatization by heating, but not actually derivatized. Reductions in water retention are observed from about 10% to about 50%, and preferably about 15% to about 45%, of the absorption capacity observed prior to the derivatization step. Water retention is measured in terms of ml H_2O/g material at standard

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temperature and pressure. When ammonia vapor retention is
used as a reference, the absorption of water vapor into the
derivatized material is no more than about 20% to about 80%
that of ammonia. Often, less that 20% water is absorbed
relative to ammonia.

In contrast, both ammonia and water are irreversibly
absorbed on heat dehydrated or hydrated tectosilicate
samples which have not been treated with acid or
derivatized and exhibit retention volumes of greater than
200 ml of vapor per column-gram of aluminous tectosilicate
under the gas-solid chromatography conditions used to
measure retention volumes. Therefore, the derivatized
tectosilicates effectively absorb ammonia from wet human or
animal excreta.

The present methods are practiced by incorporation of
naturally-occurring zeolites and zeolites prepared as
described hereinabove in diapers, bedpads, blankets,
sheets, undergarments, outer clothing and the like.
Zeolites are incorporated into vapor-permeable compartments
and moisture-permeable compartments, or distributed
throughout the textile matrix.

Bedpads and disposable diapers typically consist of an
absorbent core of natural or synthetic fibers, a permeable
top or inner sheet and a liquid-impervious back or outer
sheet. While an effective amount of zeolite can be
incorporated into any of these layers, the zeolite is
preferably incorporated into the absorbent core of the
diaper or bedpad.

An effective amount of zeolite is incorporated into, or
solution-coated onto, sheets, blankets, wipes, premoistened
towelettes, disposable diaper top sheets, plastic pants
linings, cloth diapers, diaper pails and the like. Such
methods of dispersing particulate solids onto and into
fibrous substrates are known in the art.

The amount of zeolite used varies from about 1% to 50%,
preferably about 15% to 25% of the weight of the material.
The amount used may depend on the particular use, for

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example, whether the material is intended for day or night use and whether the material is intended for use by an infant or an elderly person.

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The methods of the present invention are also practiced with animals. For example, zeolites of the invention can be incorporated into animal litter, either alone or in combination with other absorbent materials. Typical animal litter consists of absorbent inorganic or organic materials such as attapulgite, vermiculite and

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calcium montmorillonite (i.e., clay), agglomerated wood dust, wood chips, dehydrated grasses, straw, or alfalfa, fly ash and the like. The present zeolites are added in an effective amount, e.g. about 5-95%, preferably about 20% to 30% or more (based on total litter weight) to inhibit

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microbial growth and ammonia formation in the litter without substantially reducing the litter's absorbent characteristics. For convenience, such litter preparations are aggregated into pellets.

The invention is further illustrated by reference

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to the following non-limiting examples.

Example 1

The following six procedures were used to modify the properties of clinoptilolite (Hector, Cal., NL Industries).

PROCEDURE A$_1$ -- MILD ACID WASH

The tectosilicate, i.e., clinoptilolite, was crushed in a jaw crusher, then pulverized in a Braun Pulverizer. The pulverized material was passed through a 50-100 mesh RoTap® sieve agitator and used to fill 2-inch diameter, 3-foot long Pyrex® tube two-thirds full. The powdered material was held in place with a glass wool plug. Forty liters of hydrochloric acid (6N) were poured through the packed column at a rate of about 9 ml/min. at 27°C. The acid-treated material was washed by flushing with three column volumes of distilled water, then air-dried. Clinoptilolite (Hector, Cal.) treated in this manner was light green and exhibits a Si:Al ratio of approximately 30.

PROCEDURE A$_2$ -- STRONG ACID WASH

The light green material 9225 g) isolated from procedure A$_1$ was placed in a 4.0 liter round bottomed flask and 2.01 of 6N HCl was added. The slurry was heated at reflux for 2.0 hours. A white mineral was recovered by vacuum filtration and washed repeatedly with deionized water. The Si:Al ratio of clinoptilolite treated in this manner was about 212.

PROCEDURE H$_1$ -- MILD HEAT TREATMENT

About 10 g of pulverized tectosilicate (clinoptilolite) was placed in a 250 ml beaker and heated to 150°C for 20 hours in a vacuum drying oven at less than 10 mm Hg. After vacuum heating, the material was stored at 150°C at ambient pressure.

PROCEDURE H$_2$ -- HIGH HEAT TREATMENT

About 10 g of pulverized tectosilicate (clinoptilolite) was placed in a quartz 250 ml beaker and

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heated at 550°C for 14 hours at ambient pressure, then transferred to a 150°C oven for storage at ambient pressure.

PROCEDURE D_1 -- SILYLATION

Pyridine was allowed to stand over potassium hydroxide pellets for 24 hours, then distilled from barium oxide and stored over 4Å molecular sieves. Toluene was refluxed over sodium metal for three days, then distilled and stored over Linde type 4Å molecular sieves. A reagent mixture of 20% pyridine, 15% dichlorodimethylsilane and 65% toluene was prepared and stored over the molecular sieves.

A 250 ml round bottomed flask equipped with magnetic stirring, a reflux condenser and argon inlet was flushed with dry argon and charged with 10 g of pulverized tectosilicate followed by addition of 100 ml of the reagent mixture described hereinabove. The resultant slurry was refluxed for 20 hours. After reaction, the acid-treated tectosilicate material was isolated by filtration and washed with dry toluene and methanol. The material was refluxed for at least two hours in methanol, recovered by filtration and stored under ambient conditions.

PROCEDURE D_2 -- METHYLATION

About 5.0 g of pulverized material was placed in a steel bomb with about 50 ml of methanol. The bomb was sealed and heated to 220°C for 4-12 hours. The bomb was cooled to 25°C and the material recovered by filtration.

Example 2

DETERMINATION OF GAS RETENTION VOLUMES

The treated, pulverized tectosilicate was vacuum-packed into a silylated glass column (0.125 inch inner diameter, 0.25 inch outer diameter) and held in by plugs of silylated glass wool. The column was inserted into the oven of a gas chromatograph. The injector port was maintained at 200°C, the detector oven at 250°C and the column maintained at an initial conditioning temperature of 45°-50°C for 10-30 minutes. The detector filament current was held at 150mA and the carrier gas (He) inlet pressure was 60 psi. Gas injections (75-125 μl) were made at 407 psi above ambient pressure and liquid injections were of 1-2 μl. Water and ammonia retention volumes were measured at a column temperature of 200°C. Under these conditions, ammonia was irreversibly absorbed. Results were expressed as K (ml of gas absorbed/g of absorbent at STP).

The properties of a number of modified Hector, California clinoptilolites prepared by various combinations of the procedures described above are summarized in Table I. In all cases the procedures were performed or omitted in the order indicated. The silicon:aluminum ratios were determined by energy-dispersive X-ray spectrometry (Tracor spectrace model 440, Tracor-Northern 2000 Analyzer) with data reduction accomplished using the program Super ML, Tracor X-Ray, Inc.

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TABLE I

Sample	Treatment[1]	$K(H_2O)$[2]	Si/Al	Total Carbon Analysis (%)[5]
1	none	>200	10.00	0.28
2	$A_2H_1D_2$	21	High[3]	0.69
3	$A_2H_1D_1$	26	High[3]	0.62
4	$A_2H_1D_0$	36	211.67	0.12
5	$A_1H_1D_2$	58	39.58	0.52
6	$A_1H_2D_0$	59	6.93[4]	0.30
7	$A_1H_2D_1$	64	32.66	0.63
8	$A_1H_1D_1$	79	33.00	2.37
9	$A_1H_1D_0$	93	34.43	0.38
10	$A_1H_0D_2$	129	33.56	0.29
11	$A_1H_0D_0$	>200	10.82[4]	N.T.
12	$A_1H_0D_1$	>250	39.01	2.90
13	$A_1H_2D_2$	>570	6.8[4]	0.36

[1]Treatments A_1, A_2, H_1, H_2, D_1, and D_2 are described in
    Example 1. D_0 treatment indicates no derivatization; H_0
    treatment indicates no heat treatment.
[2]ml/g at STP: $K(NH_3)$ was >200 in all cases.
[3]Lattice Al not detected in these materials.
[4]Anomalous results probably due to operator error.
[5]Galbraith Laboratories, Inc., Knoxville, Tenn.

     Table I illustrates that mild or strong acid
washes followed by high or low temperature heating
significantly increases the hydrophobicity of the
tectosilicate even without a further derivatization step.
The effect was most pronounced in the case of samples
washed with strong acid, then heated at 150°C. ($A_2H_1D_0$,
Sample 4). However, samples 3 and 2 demonstrate that a
further significant increase in hydrophobicity can be
attained by silylation or methylation, respectively, of
this material. The total percent carbon was also increased
in these samples by over 400% in each case. Likewise, an
increase in hydrophobicity was observed in the case of the
silylation (Sample 8) or methylation (Sample 5) of the
material of Sample 9, which had been subjected to the mild
acid wash and then to 150°C heating. The greater affinity
for water observed for these samples, as opposed to samples
3 and 2, was thought to reflect the presence of more
reactive sites, i.e., silyl nests, in the latter two

materials, which had been exposed to stronger dealumination conditions.

Mild heat treatment before derivatization retains lattice structure and minimizes collapse of hydroxyl or other reactive sites. Sample 6 was made by mild acid treatment but high heat treatment. Derivation of sample 6 by silylation and methylation formed samples 7 and 13, respectively. However, samples 7 and 13 exhibit little or no increase in hydrophobicity relative to sample 6, indicating derivatization was ineffective. A comparison of samples 8 and 7, with 9 and 6 indicates that, for otherwise equivalently-prepared samples, a high heat treatment (H_2) results in a significantly lower carbon incorporation when either methylation or silylation was attempted. While not wishing to be limited, poor derivatization may be due to the collapse of hydroxyl nests or other reactive sites by high heat treatment.

Silylation of material which had been acid washed but not dehydrated by heat (sample 12) failed to increase the hydrophobicity of the material of sample 11, possibly due to the blockage of reactive sites by water of hydration. Methylation of the same material caused a moderate increase in hydrophobicity (sample 10). Thus, the activated nests following acid treatment were best produced and preserved by mild heat treatment.

The hydrophobic derivatized materials of samples 3 and 2 possessed no detectable lattice aluminum by X-ray fluoroscopy, a negative result also expected and observed in the case of silicalite® (Union Carbide). This provides confirmation that the strong acid wash conditions were effective to remove lattice aluminum and to produce reactive hydroxyl-containing nests that were available for derivatization. Although removal of lattice aluminum is, by itself, adequate to significantly increase the hydrophobicity of the clinoptilolite, and, in fact, was the major contributor to the hydrophobic properties involved, it was apparent from samples 8, 3, 5 and 2 that the

hydrophobic properties were optimized, for this set of
treatment variables, by further silylation or methylation.
Significant hydrophobic affects are generally observed in
both derivatized and nonderivatized materials when the

5   Si:Al ratio exceeds about 25.

The hydrophobic materials of samples 8, 3, 5 and 2
would be expected to absorb significant amounts of ammonia
from wet human or animal excreta, and to do so more
effectively than any material employed heretofore, such as

10   nonderivatized tectosilicates, phyllosilicate clays, silica
gel and the like.

Example 3

Inhibition of Microbial Growth by Derivatized Zeolites

Materials:

Three dried cultures of *E. coli* (ATCC 25922), *S. aureus* (ATCC 6538) and *S. typhimurium* (ATCC 14028) were rehydrated with four ml of Difco's Brain Heart Infusion broth (BHI) and incubated at 37°C for twenty hours. Standard plates containing Difco's Tryptic Soy Agar (TSA) were prepared for spread plating. A single tube of 4.5 ml nutrient broth with no zeolite was prepared as a positive control. Two sets of six tubes containing 4.5 ml nutrient broth were prepared for quantifying bacteria treated with each of zeolite DCDMS (dichlorodimethylsilane derivatized clinoptilolite), ZeoLog-MeTE (methyltriethoxy derivatized clinoptilolite) and ZeoLog-METH (methanol derivatized clinoptilolite). Six of these tubes were to be used for testing how bacteria responded to zeolite treatment; the other six tubes were to be used for pH measurement on days two and three. Six rows of ten tubes containing 4.5 ml 0.1% Beef Peptone Water (BPW) were also prepared for quantifying of bacteria treated with zeolite by serial dilution. A similar series of ten tubes was also prepared for titer of the positive control which would receive no zeolite.

Methods:

The twenty hour cultures were collected from the incubator and a growth initiation titer (GIT) was performed by plating 0.1 ml of 10^{-6} to 10^{-10} dilutions on TSA.

5   To determine the effect of zeolite on survival of a bacterial species, 4.5 ml of a 45 mg zeolite/Ml nutrient broth solution was placed in a tube and 0.5 ml of a 10^{-4} dilution of bacterial culture was added. This tube was designated the original 10^{-1} dilution tube. This original

10   10^{-1} solution was serially diluted by sequential placement of 0.5 ml into tubes containing 4.5 ml of a 45 mg zeolite/Ml nutrient broth to a final dilution of 10^{-6}. The 0.5 ml removed from the original (10^{-1}) tube was replaced with 0.5 ml of 45 mg zeolite/Ml nutrient broth so that each

15   tube in the series would have the same volume. A similar series of bacterial dilutions were made in nutrient broth containing no zeolite to create a positive control. The set of six zeolite-nutrient broth tubes to be used for measuring the effect of zeolite on pH received no bacteria.

20   All tubes were placed in a 37°C shaking incubator for twenty hours.

The next day, the tubes and the GIT plates were taken out of the incubator. Ten fold dilutions were made of each zeolite treatment culture and 0.1 ml of these

25   dilutions were spread plated on TSA plates. The plates were incubated for twenty hours and the number of colonies were counted. The pH of the uninoculated tubes was recorded.

30   **Results:**

Zeolites are effective inhibitors of bacterial cell growth. For example, as illustrated in Fig. 4, the cell growth of Staphlococcus aureus was completely inhibited by a large range of concentrations of a clinoptilolite which

35   has been derivatized with methanol (MeOH Zeolog, also known as ZeoLog-METH). In particular, the initial Staphlococcus aureus titer of 9.6 X 10^8 cfu/ml was reduced by 6 X 10^8

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cfu/ml when using dilutions of this 45 mg/ml zeolite suspension which ranged between 10^{-1} and 10^{-5}. Similarly, clinoptilolite derivatized with dichlorodimethylsilane completely inhibited _Escherichia coli_ cell growth at 10^{-1} to 10^{-2} dilutions of a 45 mg/ml suspension of the derivatized clinoptilolite (see Fig. 5); and clinoptilolite derivatized with methyltriethoxy inhibited _Salmonella typhimurium_ cell growth by up to 4 X 10^5 cfu/ml when a 10^{-4} dilution of the 45 mg/ml zeolite suspension was used (see Fig. 8). Hence, zeolites inhibit cell growth of all the types of bacteria which were tested.

However, for one bacterial species, one type of zeolite derivative may be more effective at one concentration than another concentration. Thus, clinoptilolite which has been derivatized with methanol (MeOH Zeolog, also known as ZeoLog-Meth) was highly effective at wide range of concentrations against _Staphlococcus aureus_ (see Fig. 4). But a dilution of 10^{-1} of a 45 mg/ml suspension of clinoptilolite derivatized with methyltriethoxy groups (ZeoLog-MeTE) was needed to cause strong inhibition of _Staphlococcus aureus_ cell growth. Similarly, while a dichlorodimethylsilane derivative of clinoptilolite caused little inhibition of _Salmonella typhimurium_ cell growth (see Fig. 7) in the range of concentrations which were tested, a methyltriethoxy derivative of clinoptilolite was an effective inhibitor of _Salmonella typhimurium_ (see Fig. 8) at 10^{-3} and 10^{-4} dilutions (see Fig. 8). Similarly, a dichloro-dimethylsilane zeolite derivative was an effective inhibitor of _Escherichia coli_ over a broad range of concentrations (see Fig. 5) while a methyltriethoxy derivative was strongly inhibitory only at 10^{-1} and 10^{-2} dilutions (Fig. 6). Thus, methanol derivatized zeolites are strong inhibitors of _Staphlococcus aureus_, while methyltriethoxy derivatized zeolites are effective against _Salmonella typhimurium_, and dichlorodimethylsilane zeolites are optimally effective against _Escherichia coli_.

Example 4

Total Bacteriological Population in the Lungs of Chickens Fed Zeolites Derivatized with DCDMS

Methods:

Batches of freshly hatched chicks were raised on feed containing clinoptilolite derivatized with DCDMS, in the amounts specified in Table II.

TABLE II

Treatment[1]	Amount zeolite in feed
1	50 mg/lb DCDMS zeolite
2	250 mg/lb DCDMS zeolite
3a	control: 125 mg/lb aluminated and nonderivatized zeolite
3b	control: 125 mg/lb dealuminated but nonderivatized zeolite)
4	500 mg/lb DCDMS zeolite
5	125 mg/lb DCDMS zeolite

This feed was the only feed given to these chickens from the time of hatching until slaughter, which occurred at about 7-8 weeks of age. The chickens were examined by a veterinarian for symptoms of disease, malnutrition and poor growth. The histopathology of the chickens' hearts, livers, spleens, kidneys, trachea and lungs were also observed (see Tables III and IV). While no overall toxic effects of this feeding regimen were observed, some lesions were observed in the trachea and lungs which were likely due to irritation or inhalation of foreign material (see Table IV). The number of colony forming units per gram of lung tissue was determined to ascertain whether one group of chickens had an increased propensity for lung infections, e.g. bacterial pneumonia.

Results:

No negative effects upon the chickens were observed.

TABLE III

HISTOPATHOLOGY REPORT: ZEOLITE EXPERIMENT #1

Heart - In most birds there were no alterations. A few had scattered and small infiltrations of lymphocytes between muscle bundles. Intimal proliferation in small arteries occurred in two birds.

Liver - The usual focal collections of lymphocytes were present in most of the livers. These foci were scattered in the parenchyma and in portal areas. In some birds the lymphocytes were mixed with immature granulocytes. The epithelium of occasional bile ducts was hypertrophic.

Spleen - No remarkable lesions.

Kidney - Focal collections of lymphocytes, in follicular formation or diffuse infiltration, were scattered in the cortex or medulla of several birds.

Trachea - An inflammation of varying severity involved the trachea of most birds. These lesions were characterized by infiltration of lymphocytes in the mucosa. The infiltrations were diffuse or follicular and involved focal are diffuse areas of the trachea. Little exudate was present in the lumen. The cilia were not involved.

Lung - Nodular collections of lymphocytes occurred in the mucosa of bronchi in all lungs. Granulomas, consisting of necrotic centers with surrounding lymphocytes, heterophiles and multinucleated giant cells, were present in the lumina of bronchi in many birds. Bacterial colonies were often present in the granulomas and mycelia were identified in two birds. Crystalline foreign material was

observed in the lesions of one bird. Occasionally
the infection had spread in the peribronchial
tissue resulting in areas of pneumonia.

Foci of cartilage and/or bone were scattered in the
5   parenchyma of many birds.

TABLE IV

SUMMARY OF LESIONS

		Tracheitis*	Lung*			
		Av. Score **	Granuloma	Bact	Fungus	Pneumonia
Tmt 1	12/24	0.51	5/24	1/24	1/24	0/24
Tmt 2	20/24	1.50	12/23	4/23	0/23	10/23
Tmt 3	18/24	1.25	10/24	5/24	0/24	6/24
Tmt 4	12/24	0.83	9/24	1/24	1/24	0/24
Tmt 5	17/24	0.95	6/24	4/24	0/24	6/24

*   Number/Total

**Lesion Score (severity) 0 normal, 4 most severe.

There was no evidence of toxicity in these birds.

Only the lesions of the respiratory system were
possibly related to the experiment. The lymphocytic
infiltrations occurring in the liver, spleen and kidney are
commonly observed in birds in the field. The occasional
hyperplasia of the bile duct epithelium was not associated
with the experimental treatment. Peri-ductal infiltrations of
heterophiles are observed occasionally in field birds.

The lesions of the trachea and lung were those of
irritation and/or inhalation of unidentified foreign material.
Tracheitis was possibly the result of ammonia levels. The
location of the granulomas in the lumina of bronchi suggested
the lesions were related to inhalation. The lesions appeared
to arise from some material or organism which had lodged on
the mucosal surface. Infection in some cases had spread to
adjacent areas of the lung.

Chickens were tested to determine whether feeding derivatized zeolites increased or decreased bacterial infections in the lung. The treatment regimen was provided in Table II. Table V summarizes the results.

TABLE V
Effect of Feeding Chickens Clinoptilolite derivatized with DCDMS

TREATMENT[1]	REPL[2]	LUNG CFU/MAC[3]
1-50 mg/lb	1	3000/gm
1-50 mg/lb	2	12000/gm
2-250 mg/lb	1	450/gm
2-250 mg/lb	2	800/gm
3a-control[4]	1	60000/gm
3b-control[5]	2	15000/gm
4-500 mg/lb	1	115/gm
4-500 mg/lb	2	180/gm
5-125 mg/lb	1	1500/gm
5-125 mg/lb	2	12000/gm

[1] Amount DCDMS-derivatized clinoptilolite in feed.
[2] Replicate number.
[3] Number of colony forming units observed on a MacConkey agar
    plates per gram of lung tissue.
[4] 3a Control -- chickens were fed 125 mg/lb aluminated and
    nonderivatized zeolite.
[5] 3b Control -- chickens were fed 125 mg/lb dealuminated and
    nonderivatized zeolite.

As illustrated in Table V, feeding increasing amounts of dealuminated, derivatized zeolite to chickens decreases the number of colony forming units (CFU) in the lung. Chickens fed aluminated zeolites had 60,000 CFU per gram lung tissue. Chickens fed dealuminated zeolites had five-fold fewer CFU. Hence, dealumination of zeolites fed to chickens causes a

reduction in bacterial infection, even when the zeolites are not derivatized.

Derivatization provides even greater anti-bacterial effect. Hence, the CFU per gram of lung tissue decreases by at least about twenty-fold, from 3000-12000 when chickens are fed 50 mg derivatized zeolite per pound of feed, to 115-180 when chickens are fed 500 mg derivatized zeolite per pound of feed. Feeding derivatized zeolite therefore protected chickens against lung infection.

WHAT IS CLAIMED:

1. A method for preventing a microorganism from growing on a surface or in an absorbent material which method comprises: incorporating an effective amount of zeolite onto said surface or into said absorbent material to inhibit said microorganism from growing.

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2. The method of Claim 1 wherein said zeolite is a silaceous zeolite.

3. The method of Claim 1 wherein said zeolite is analcite, brewsterite, habazite, clinoptilolite, dachiardite, erionite, faujasite, ferrierite, flakite, gmelinite, harmotone, heulandite, leucite, levynite, mesolite, mordenite, natrolite, nepheline, noselite, paulingite, phillipsite, ptilolite, scolecite, stilbite, or yugawaralite.

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4. The method of Claim 1 wherein said zeolite is clinoptilolite.

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5. The method of Claim 1 wherein said zeolite is aluminous tectosilicate.

6. The method of Claim 1 wherein said zeolite is hydrophobic microporous crystalline tectosilicate of regular geometry having aluminum-free sites in a silaceous lattice;
    wherein said sites have about 1 to about 4 associated moieties of the formula $\equiv$SiOR; and
    wherein each R is a substituent that is a weaker point electric source than aluminum.

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7. The method of Claim 6 wherein each R is a substituent that is a weaker point electric source than hydroxy.

8. The method of Claim 6 wherein each R is selected from the group consisting of C_1-C_4 alkyl, C_1-C_4 alkoxy, C_1-C_4 acyl, and SiR'_nX_p, wherein:
    each R' is selected from the group consisting of C_1-C_4 alkyl, cyclolalkyl, aryl, C_1-C_4 acyl, and aralkyl;
    each X is a halogen or a C_1-C_4 alkoxy;
    each n is an integer of 0 to 3; and
    each p is 3 - n.

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9. The method of Claim 6 wherein a aluminum-free site is bridged by 1 or 2 SiR'_q, wherein

each R' is selected from the group consisting of C_1-C_4 alkyl, cyclolalkyl, aryl, C_1-C_4 acyl, and aralkyl.

10. The method of Claim 1 wherein said zeolite is a dichlorodimethyl silane zeolite, ZeoLog-MeTE, ZeoPhob™, ZeoLog™, ZeoLogCN-METHANOL™, Zeolite A, Zeolite B, Zeolite D, Zeolite E, Zeolite F, Zeolite H, Zeolite J, Zeolite KG, Zeolite L, Zeolite M, Zeolite O, Zeolite Q, Zeolite R, Zeolite S, Zeolite T, Zeolite W, Zeolite X, Zeolite Y, and Zeolite Z.

11. The method of Claim 1 wherein said surface is skin, wall, floor, countertop, or wallcovering.

12. The method of Claim 1 wherein said absorbent material is a diaper, clothing, bedding, bedpad, surgical apparel, or surgical mask.

13. The method of Claim 1 wherein said effective amount of zeolite is about 1% to about 50% of the weight of the absorbent material.

14. The method of Claim 1 wherein said effective amount of zeolite is about 15% to about 25% of the weight of the absorbent material.

15. The method of Claim 1 wherein said microorganism is a gram negative species of bacteria.

16. The method of Claim 15 wherein said gram negative species is an Enterobacteriaceae.

17. The method of Claim 16 wherein said Enterobacteriaceae is a Proteus.

18. The method of Claim 17 wherein said Proteus is Proteus mirabilis, Proteus vulgaris, Proteus penneri or Proteus myxofaciens.

19. The method of Claim 16 wherein said Enterobacteriaceae is a Klebsiella, Providencia, Yersinia, Erwinia, Enterobacter, Salmonella, Serratia, Aerobacter and Escherichia.

20. The method of Claim 1 wherein said microorganism is Escherichia coli or Staphylococcus aureous.

21. The method of Claim 16 wherein said gram negative bacteria is Pseudomonas, Shegella, Vibrio, Aeromonas, or Campylobacter.

22.   The method of Claim 1 wherein said microorganism is a gram positive microorganism.

23.   The method of Claim 22 wherein said microorganism is <u>Streptococcus</u>, <u>Staphylococcus</u>, <u>Lactobacillus</u>, <u>Micrococcus</u> or <u>Moraxella</u>-<u>Neisseria</u>.

A.   CLASSIFICATION OF SUBJECT MATTER

IPC(6)   :A61K 33/06
US CL   :424/684

According to International Patent Classification (IPC) or to both national classification and IPC

B.   FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S.   :   424/684

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C.   DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Chemical Abstracts, Volume 124 No185635, issued 1996, Kawai et al." Long-lasting film-forming disinfectant compositions and sterilization using them". See the entire Abstract.	1-23

☐   Further documents are listed in the continuation of Box C.   ☐   See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 MAY 1997	2 4 JUN 1997

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